NewbornScreeningNews

A NEWSLETTER OF THE NEWBORN SCREENING PROGRAM AND THE NEWBORN SCREENING LABORATORY

Fall 2005

Revealing Biotinidase

Testing Starts January 2006

What to Watch For

Lab Processes

Do's and Don'ts

Genetics

Biotinidase Deficiency

By Nicola Longo, MD PhD

Biotinidase deficiency is an autosomal recessive disorder caused by deficiency of an enzyme required for biotin recycling and extraction from common sources. Biotin is attached to the epsilon amino group of lysine within proteins either ingested with the diet or recycled from endogenous sources. In the body, free biotin is released by the action of biotinidase and can be attached to different enzymes for which it is an essential cofactor (acetyl CoA carboxylase, propionyl CoA carboxylase, pyruvate carboxylase, and β-methylcrotonyl CoA carboxylase) by the action of holocarboxylase synthase (Fig. 1).

Profound biotinidase deficiency (<10% of normal activity) if not treated causes neurological and cutaneous findings that include seizures, hypotonia, and rash, often accompanied by hyperventilation, laryngeal stridor, and apnea. Children might present shortly after birth or later in life with these symptoms. Older children may also have alopecia, ataxia, developmental delay, neurosensory hearing loss, optic atrophy, and recurrent infections.

Patients with partial biotinidase deficiency (10-30% of normal) may have hypotonia, skin rash, and hair loss, particularly during times of stress. Based on the results of worldwide screening of biotinidase deficiency, the incidence of the disorder is one in 130,000 for profound biotinidase deficiency; one in 110,000 for partial biotinidase deficiency; and one in 60,000 for the combined incidence of profound and partial biotinidase deficiency. Carrier frequency in the general population is about one in 120.

Laboratory studies in most affected patients might reveal metabolic acidosis with mild hyperammonemia. Multiple abnormal organic acids can be detected in urine reflecting deficiency of multiple carboxylases. The absence of organic aciduria or metabolic acidosis does not exclude the diagnosis of biotinidase deficiency in a symptomatic child. In fact, biotinidase deficiency in certain patients affects selectively the brain, with neurological manifestations, vision and hearing loss, but no metabolic abnormalities. These children might also lack cutaneous manifestations of the disease.

All symptomatic children improve when treated with 5 to 10 mg of oral biotin per day. In some treated children all symptoms resolve, whereas in others some deficits are irreversible. For this reason, early initiation of therapy is essential.

Newborn screening will detect profound and partial biotinidase deficiency. In positive children, the results of screening need to be confirmed by enzyme assay performed in blood. Siblings of affected patients need to be tested for the disease, because some patients present late and might still be in the asymptomatic phase.

Biotin supplements (5 mg BID) can be initiated while awaiting confirmation of the screening results if the child is symptomatic.

Diagnosis can be confirmed by DNA analysis that detects about 60% of disease-causing mutations. Biotin needs to be continued for life. This is sometimes a problem, because patients do so well that they (and their family) tend to forget that they have this disease.

Patients need to be evaluated periodically in the metabolic clinic where blood tests might be ordered to assure compliance with therapy. During that time, a referral might be recommended for periodical evaluations with audiology or ophthalmology.

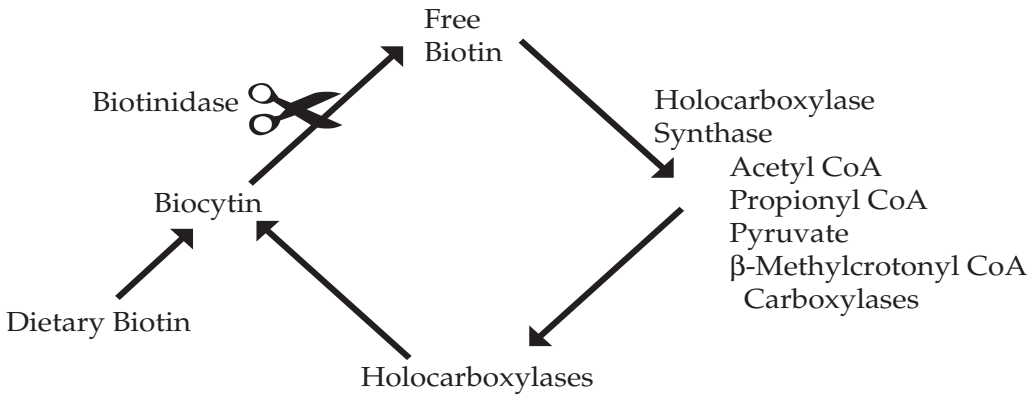

Fig. 1. Biotinidase function. Biotinidase releases free biotin from ingested or recycled proteins. Biotin is then attached to different carboxylases by another enzyme (holocarboxylase synthase). With biotinidase deficiency

Key Points of Biotinidase

- Affects 1 in130,000 for profound, 1 in 110,000 for partial and 1 in 60,000 for combined.
- If not treated causes neurological and cutaneous findings such as seizures, hypotonia and rash often accompanied by hyperventilation, laryngeal stridor and apnea.
- Older children may also have alopecia, ataxia, developmental delay, neurosensory hearing loss, optic atrophy and recurrent infections.
- Laboratory studies in most affected patients might reveal metabolic acidosis with mild hyperammonemia.
- In certain patients, the brain is selectively affected with neurological manifestations such as, vision and hearing loss, but no metabolic abnormalities.

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Wolf B (1995) Disorders of biotin metabolism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The Metabolic and Molecular Bases of Inherited Disease, 7 ed. McGraw-Hill, NY, pp 3151-77

Wolf B (2003) Biotinidase Deficiency: New Directions and Practical Concerns. Curr Treat Options Neurol 5:321-328

http://genetests.org/servlet/access?id=8888891&key=yZZdvpkJEkO4s&gry=INSERTGRY&fcn=y&fw=truT&filename=/glossary/profiles/biotin/details.html

What Causes Biotinidase Deficiency?

By Pilar Lenglet, MS CGC

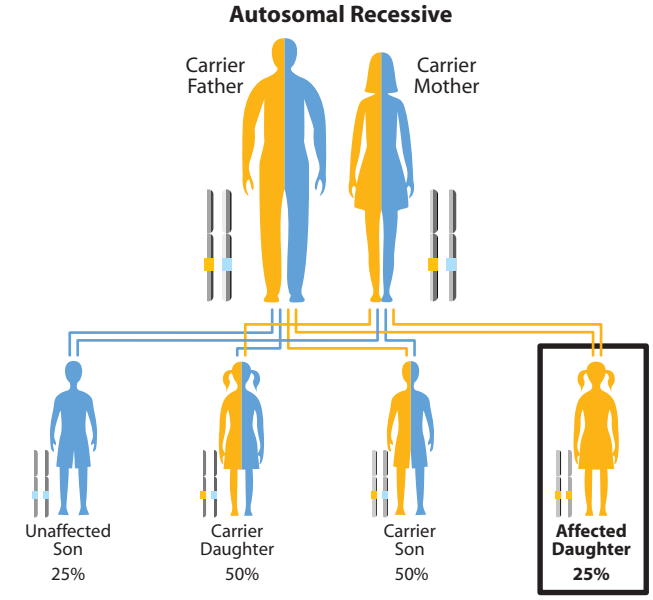
Biotinidase deficiency is an autosomal recessive condition caused by mutations in the BTD gene located on chromosome 3. BTD encodes the enzyme, biotinidase. Mutations (changes) in this gene cause the gene to not function properly, thus leading to a deficient enzyme product. Normally individuals have two normal copies of the BTD gene. Individuals with biotinidase deficiency have mutations in both copies of the gene. Individuals who have one normal gene and one abnormal gene with a mutation are called 'carriers'. Carriers have approximately 50% of enzyme activity level, yet do not have symptoms of biotinidase deficiency and do not require biotin treatment.

What are the chances of having another child with biotinidase deficiency?

Both parents of a child with biotinidase deficiency are carriers of biotinidase deficiency (one normal gene/one abnormal gene). If both parents are carriers of a condition, there is a 25% chance (1 in 4) of having another child (boy or girl) with biotinidase deficiency with each pregnancy (see picture).

What are the risks for other family members?

The chance of other family members having a child with biotinidase deficiency depends on the carrier status of both parents. The chance that someone in the general population is a carrier of biotinidase deficiency is approximately 1 in 120 (0.8%). Given this information, the individual with biotinidase deficiency has a 1 in 240 chance of having a child with this condition. Siblings of the affected child have a 2/3 chance of being a carrier. Aunts and uncles of the affected child (siblings of the parents) have a 50% chance of being a carrier. Carrier testing is possible by measuring serum biotinidase activity or by molecular genetic mutation analysis, if the mutations within the family are known.



From the State Lab

By Norm Brown, BS MT

Interpretation of Abnormal Color Development in Standards and Control

Solution Color	Interpretation
Purple (-)	Biotinidase activity is present; negative for biotinidase deficiency.
Intense Purple	This may indicate that the sample has high enzyme activity or more likely that the sample contains an aromatic amine, such as a sulfonamide. Color development of the blood spot in KPB without substrate indicates the presence of an aromatic amine. These patients should be retested when the antibiotic is no longer being administered.
Pale Purple	Very low biotinidase activity; potentially positive for partial biotinidase deficiency. Obtain an additional blood disk from the initial card and repeat the screening test.
Straw (+)	No biotinidase activity present; potentially positive for profound biotinidase deficiency. Obtain an additional blood disk from the initial card and repeat the screening test.

Biotinidase is a semi-quantitave measurement of the enzyme Biotinidase. Whole blood samples are collected by thoroughly saturating filter-paper spots on neonatal screening cards. The cards must be air dried thoroughly before being placed inside a protective covering, such as an envelope. Dried blood spots are eluted in substrate wells containing biotin-4-aminobenzoic acid. After incubation, a series of reagents are added to develop color.

Biotinidase cleaves the amide bond of the artificial substrate, B-pABA, resulting in free biotin and p-ABA. The free p-ABA is then diazotized and coupled to a naphthol reagent to produce a purple product, which can be observed. If Biotinidase is present in the sample, a color change to purple is noted, indicating the substrate has been cleaved to form biotin. The intensity of the purple color is a measure of the degree of Biotinidase activity. The absence of color, or a very pale hue of purple, suggests the absence of low level of Biotinidase activity. In the absence of biotinidase activity, p-ABA is not liberated from the substrate and color development does not occur. Sample solutions lacking biotinidase activity appear straw colored. Specimens that are found to be "Abnormal" on the test are retested in duplicate before a report is made.

Biotinidase, like other enzymes, is subject to deterioration when exposed to heat. This is most common during summer months and result in Biotinidase deficient specimens. The reporting categories that will be used are Negative (purple color), Positive (straw color) and Indeterminant (intense or pale color).

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Newsletter Design by Frank Cortese
Cover photo: © Frank Cortese
96-well microtiter transfer plates with Biotinidase reagents showing color discrimination of various intensities of purple color or the lack of color.
Photo taken with Sony© Cyber-shot 3.2 mega pixel camera.
Plate over light table and touched up using Adobe© Photoshop 6.0.

Check out the following websites for more information on Biotinidase and newborn screening.

- **Utah Department of Health, Newborn Screening Follow Up Program**
  http://health.utah.gov/newbornscreening
- **National Organization for Rare Disorders**
  http://www.rarediseases.org
- **Biotinidase Deficiency Family Support Group**
  http://biotinidasedeficiency.20m.com
- **Families.com**
  http://encyclopedias.families.com/biotinidase-deficiency-157-159-gecd
- **Biotinidase Deficiency, A Booklet for Families and Professionals**
  http://www.ccmckids.org/research/biotinidase/Biotinidase%20Deficiency%20Booklet%203-12-03.pdf

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Biotinidase

Quick Facts

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Newborn
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- Biotinidase deficiency is a rare, treatable, genetic condition in which the body cannot use dietary biotin.
- Infants may be identified by newborn screening before symptoms develop or a diagnosis may be made in an older infant or child who has been experiencing some of the following problems:
  seizures, hair loss, skin rash, developmental delay and hearing loss.
- Lifelong treatment with prescribed doses of biotin is very effective in preventing or improving most symptoms.
- Parents who have a child with biotinidase deficiency have a 1 in 4 or 25% chance with each preganancy of having another child with biotinidase deficiency.
- Other family members may also carry the gene for biotinidase deficiency.
- Carrier testing and prenatal testing is available.
- It is possible to determine the mutation(s) in the biotinidase gene of your child that causes biotinidase deficiency (about 60% of disease-causing mutations).